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Crosstalk Between Oxidative Stress, Autophagy and Cell Death — Pathogenesis of Autoimmune Disease

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Additional information is available at the end of the chapter

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1. Introduction

Increased free radical formation and altered redox state are fundamentally important in autoimmune diseases pathogenesis. Free radicals are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS) in mitochondria, cellular membranes, and the endoplasmic reticulum membrane as physiological responses to a variety of internal and external stress. These free radicals play both beneficial and deleterious roles in our body's defense system [1]. ROS/RNS are beneficial to our body at low/moderate concentrations via activation of redox-sensitive signaling pathways, phagocytosis of infected cells, induction of mitogenic responses for wound healing, and clearance of abnormal or aging cells as a part of an important surveillance mechanism [2-4].

Autophagy is a persistent homeostatic process in which certain cellular components are engulfed by autophagosomes, and are subsequently degraded in order to produce energy, or preserve viability and homeostasis. Autophagy breaks down compromised cellular components, such as damaged organelles and aggregated proteins, whose deposition within cells can lead to toxic effects resulting in destruction of tissues, organisms, and biological systems [5]. Alterations in autophagic cycle rate (flux), which initiates with formation of phagophore and terminates with degradation of autophagosome cargo after its fusion with lysosome, are generally observed in response to stress [6]. In most cases, autophagy primarily serves an adaptive role to protect organisms against diverse pathologies, including infections, cancer, neurodegeneration, aging, and heart disease. However, in certain experimental settings, autophagy may be deleterious [7]. Evidence from genetic, cell biology and lupus animal model

studies suggests a pivotal role of autophagy in mediating the development of systemic lupus erythematosus (SLE) [8]. The current chapter will focus on the following areas: (i) molecular mechanism(s) by which ROS/RNS generate; (ii) redox signaling and altered autophagic flux rates; (iii) the role of autophagy as a cell death progression or survival mechanism in response to oxidative stress; and (iv) modulation of autophagy in antioxidant response relative to autoimmune disease. Attention is specifically focused on understanding the molecular basis of events by which autophagy is fine tuned by oxidation/reduction events in autoimmune disease, especially SLE. Understanding the intricate relationships between oxidative stress with both apoptosis and autophagy in SLE pathogenesis could be critical in elucidating key pathogenic mechanisms, possibly leading to novel interventions for clinical disease management.

2. Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) is a general term for chemical species which are generated from incomplete oxygen reduction. These are short lived molecules formed as a natural byproduct of normal cellular metabolism and have dual role; deleterious and beneficial effect in our body. ROS comprises several oxygen ion radicals such as superoxide anion radical ($O_2^{\cdot-}$), peroxy radical (ROO^{\cdot}), extremely reactive hydroxyl radical (OH^{\cdot}), peroxide (hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), and perhydroxyl radical (HO_2^{\cdot}). Sources and modes of action of various reactive species are shown in Table 1 Beneficial role of ROS occur at low to moderate concentration that can be demonstrated by several important role of ROS in cell signaling and in defense against infectious agents and induction of a mitogenic response and immune functions.

When the ROS productions are not scavenged sufficiently by antioxidant system, it causes oxidative stress and shows harmful effect. These dangerous reactive species are formed by (1) enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) [9] or nitric oxide synthase [10], (2) non-enzymatic reactions through the mitochondrial electron transport chain [11], or (3) reduced transition metals [12]. ROS can also interact with nitric oxide (NO), whose expression is usually accompanied by inflammatory lesions. The product of NO synthases results in conversion of NO to various reactive nitrogen species (RNS.), including nitrosonium cation (NO^+), nitroxyl anion (NO^-), and peroxynitrite ($ONOO^-$) (Table 1).

Reactive species	Sources	Modes of action
Super oxide (SO)	NADPH oxidase	Oxidative stress Redox signaling
	Xanthine oxidase	
	Complex I/Complex III (mitochondria)	
	5-lipoxygenase, Cyclooxygenase	
	Uncoupled nitric oxide synthase	
Hydrogen peroxide (H_2O_2)	Peroxisomes	Redox signaling

Reactive species	Sources	Modes of action
	Super oxide dismutase	Oxidative stress
Hydroxyl radical (OH)	Fenton reaction	Oxidative stress
	Haber-Weiss reaction	
Hypochlorous acid (HOCl)	Myeloperoxidase (MPO)	Chlorination Oxidative stress
Nitric oxide (NO)	Nitric oxide synthase	Nitrosylation of metal centers
		Redox signaling
Peroxynitrite (ONOO-)	Reaction between NO and SO	Nitration, oxidation Oxidative stress
Nitrogen dioxide radical (NO ₂)	Reaction between NO and O ₂	Nitration, oxidation
		Oxidative stress
Dinitrogen trioxide (N ₂ O ₃)	Reaction between NO and NO ₂	Nitrosation
		Nitrosative stress

Table 1. List of critical reactive oxygen and nitrogen species, their sources, and modes of action.

3. Sources of oxygen radicals and their scavengers

Reactive oxygen species mainly originate from mitochondria and blood compartment, including lymphocytes from SLE patients (Table 1). ROS show hyperpolarization of mitochondria and activated T lymphocytes [13, 14]. We and others have shown an increased production of ROS or diminished levels of intracellular reduced glutathione in various blood compartments (RBC, lymphocytes) from SLE patients [15, 16]. Beside these important source of ROS, these reactive oxygen species are also produced other sources such as NADPH oxidase (NOX enzyme) in activated phagocytes [9], and minor amount from endothelial cells, macrophage and polymononuclear cells [16-18], lysosome (myeloperoxidase undergoes a complex array of redox transformations and produces HOCl), and microsomes [19, 20]. Hydrogen peroxide is formed through the dismutation of O₂^{•-} catalyzed by enzyme superoxide dismutase, and is also produced via action of several other oxidase enzymes (e.g., amino acid oxidases) (Figure 1). Beside the role of ROS in cell damage, it is also involved in inflammation generation by activation of nuclear transcription factor NF-κB, a master pathway of inflammation generation. Once, the NF-κB is activated it leads to upregulation of pro-inflammatory cytokines and leukocyte adhesion molecules.

The most harmful ROS are hydroxyl radicals, OH[•] and O₂^{•-}. Superoxide ion is converted into stable non-radical hydrogen peroxide by SOD enzyme which is then reduced by following 3 mechanisms. In a detoxification mechanism, catalase and glutathione peroxidase convert H₂O₂ to H₂O + O₂, this is considered as a detoxification mechanism. In the next mechanism, H₂O₂ is converted by myeloperoxidase (MPO) in neutrophils to hypochlorous acid (HOCl). This appears to be a mechanism for a physiological toxic agent, since HOCl is a strong oxidant

that acts as a bactericidal agent in phagocytic cells. Reaction of HOCl with H_2O_2 yields singlet oxygen (1O_2) and water. The biological significance of singlet oxygen is unclear. In the last mechanism, H_2O_2 is converted in a spontaneous reaction catalyzed by Fe^{2+} (Fenton reaction) to the highly reactive hydroxyl radical ($\cdot OH$). As the hydroxyl radical cannot be removed without causing oxidative damage, it reacts rapidly with biological molecules such as lipid, protein and DNA, triggering severe consequences in SLE pathogenesis [21-24].

4. Mechanism of oxidative damage

Reactive oxygen species, specifically the hydroxyl radical, respond with lipid layers and produces receptive aldehydes, including malondialdehyde and 4-hydroxy-2-nonenal (HNE), in three phase responses. It can "spread" oxidative damage through flow in SLE patients [25]. In the start stage an essential receptive radical concentrates a hydrogen atom from a methylene gathering to begin peroxidation. These outcomes in the development of a conjugated diene, leaving an unpaired electron on the carbon. Carbon-focused unsaturated fat radicals consolidate with sub-atomic oxygen in the proliferation stage, yielding profoundly receptive peroxy radicals that respond with an alternate lipid particle to structure hydroperoxides. Peroxy radicals are fit for creating new unsaturated fat radicals, bringing about a radical chain response. The course of lipid peroxidation bring about a mixed bag of unsafe final items including conjugated dienes, isoprostanes, 4-hydroxy-2-nonenal (HNE), HNE-altered proteins, malondialdehyde (MDA), MDA-adjusted proteins, protein-bound acrolein and oxHDL, which are associated with SLE disease activity [26-28]. In addition to the involvement of ROS in lipid peroxidation, it can modify both structure and function of proteins in SLE patients [21, 29]. Metal-catalyzed protein oxidation brings about expansion of carbonyl gatherings, cross-connecting, or discontinuity of proteins. Lipid (peroxidation) aldehydes can respond with sulfhydryl (cysteine) or essential amino acids (histidine, lysine). Essentially, adjustment of individual nucleotide bases, single-strand breaks and cross-connecting are regular impacts of ROS on nucleic acids. All these communications of ROS with protein, lipid and nucleic corrosive prompts the development of adducts items which are exceedingly immunogenic and are perceived as an outside molecule to our body, which may be included in arrangement of pathogenic auto-immune response in SLE [30, 31].

5. Antioxidant defense system

The impact of ROS is restricted by the presence of various regulatory systems in aerobic organisms to maintain redox homeostasis. A comparatively large number of compounds possess some measurement of antioxidant activities. They keep up a harmony between the generation and scavenger of ROS, and shield the cell from oxidative damage [32, 33]. Antioxidant enzymes comprise SOD (superoxide dismutase), CAT (catalase) and glutathione related enzymes; GPx (glutathione peroxidase), GR (glutathione reductase) and GST (Glutathione S-

transferase), while non-enzymatic scavengers include vitamins E, C and A and thiol containing compounds such as glutathione [34].

Reduced glutathione (L- γ -glutamyl-L-cysteinylglycine) is the most prevalent cellular thiol and the most abundant low molecular weight peptide present in all cells [35]. GSH has an amazingly essential role as a reductant in the very oxidizing environment of the erythrocyte. GSH levels in human tissues ordinarily run from 0.1 to 10 mM, and are most gathered in the liver (upto 10 mM), spleen, kidneys, lens, erythrocytes and leucocytes [36]. In cell, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the oxidized form (GSSG). Reduced form of glutathione is required for many critical cellular processes and plays a cardinal role in cell maintenance and regulation of the thiol-redox [37]. Thus the ratio of GSH/GSSG is a significant marker for characterizing oxidative anxiety. Changes in this ratio relate with disease activity in SLE patients [15, 29]. Cellular GSH levels affect T helper cell maturation [38], T cell proliferation [39], and susceptibility to ROS secreted by inflammatory cells. Additionally, many correlations exist between immune system dysfunction and alterations in GSH levels in the cells of SLE patients. We and others have reported that GSH depletion in antigen presenting cells inhibits Th1-related cytokine production like IFN- γ and IL-12, which supports Th2- mediated humoral immune response in SLE patients [39]. Protection against oxidative damage is normally afforded by replenishing intracellular reduced glutathione through an antioxidant supplement of glutathione precursors N-acetylcysteine (NAC). Evidence from SLE patients and lupus prone mice supports the role of glutathione as antioxidant therapy to diminish oxidative stress and severity of disease. In (NZB \times NZW) F1 lupus-prone mice NAC treatment prevented the decline of glutathione, including GSSG ratios, reduced autoantibody production, development of nephritis, and prolonged survival [40]. Two pilot studies of NAC treatment in SLE patients, Tewthanom (40 SLE patients) and Perl (36 SLE patients), showed that NAC treatment is effective in reversing glutathione depletion and improving disease activity and fatigue in SLE patients [16, 41]. These studies demonstrated that intracellular depletion displays an increased in oxidative stress and replenishment of intracellular glutathione may diminish disease activity in SLE patients.

Superoxide dismutase is a metalloprotein, thought to be the first line of resistance against free radicals. It catalyzes the dismutation of superoxide radical into oxygen and hydrogen peroxide [42]. If not rummaged successfully, superoxide radicals might specifically inactivate a few proteins like CAT and GPx, which are expected to take out hydrogen peroxide from intracellular medium [43]. This enzyme is found in 3 forms in human are: SOD1 located in the cytoplasm, SOD2 in the mitochondria, and extracellular SOD3 [44]. While SOD1 is dimeric [42], SOD2 and SOD3 are tetrameric [45, 46]. SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive center. Several groups reported decreased SOD activity and formation of auto-antibodies against SOD in SLE patients [47-49]. Antibodies against SOD are reported to have a role in the inactivation of SOD enzyme during increase oxidative state are responsible to deleterious effect in SLE patients.

Catalase is generally present in peroxisomes (80%) and cytosol (20%) of all oxygen consuming cells and responsible for managing harmful hydrogen peroxide produced inside cells. It catalyzes hydrogen peroxide to water and oxygen without production of free radicals [50, 51].

CAT is most elevated in the liver, kidney and erythrocytes and low in connective tissues [52]. In the most organs (e.g., liver, kidney), catalase is found as particle found (mitochondria and peroxisomes) where it exists in soluble state in erythrocytes. Under the physiological condition, H_2O_2 is catalyzed by glutathione peroxidase (high affinity for H_2O_2) while at high concentration H_2O_2 is removed by catalase enzyme [12]. The importance of catalase enzyme in the SLE patients is well documented in SLE patients. The polymorphism catalase enzyme (-330CC genotype) showed remarkable association with several disease activities such as thrombocytopenia, renal manifestations, as well as production of anti-nRNP and anti-Scl-70 antibodies in SLE patients [53]. An elegant study by Mansour et al. reported that elevated levels of auto-antibodies against catalase in excessive oxidative stress state in SLE patients are associated with disease activity [54]. In two different studies they demonstrated that SLE patients have increased levels of IgG antibodies (Ab) against CAT in SLE patients compared to control subjects [54, 55].

Glutathione peroxidase belongs to the selenoprotein family and is one of the most important anti-oxidant enzymes in human. It catalyses hydroperoxides and protects biomembranes and cell structures from oxidative damage. [56]. This enzyme accomplishes these protections through utilization of glutathione as a reducing substrate and converting hydroperoxides to free hydrogen peroxide to water [57]. In animals studies the importance of this enzyme can be illustrated by following evidences. Glutathione peroxidase knockout mice have abnormal cardiac mitochondria and associated with increase mitochondrial ROS production and oxidative mtDNA damage.

In SLE patients, decrease activity of glutathione peroxidase enzyme has been associated with increase in oxidized redox environment in cell [47]. Since, glutathione peroxidase enzyme is also required for maintaining intracellular glutathione levels, which is key antioxidant and control oxidative stress and involved in regulating several immune functions such as apoptosis [58] and cytokine network [38] in SLE pathogenesis.

6. Oxidative-stress and autophagy

Excessive oxidative stress and altered redox signaling are most commonly known to be involved in cell death signaling cascades. However, their role in regulation of autophagy is largely unknown in autoimmune diseases. Autophagy is a persistent homeostatic process in which certain cell components are engulfed by autophagosomes and, subsequently degraded in order to produce energy or preserve cellular viability and homeostasis [59]. Autophagy breaks down compromised cellular components, such as damaged organelles and aggregated proteins. Deposition of these components within cells can lead to toxicity, resulting in destruction of tissues, organisms, and biological systems [60]. Elevated ROS causing autophagy promotes either cell survival or cell death, the fate of which depends upon the severity of stress occurring with a particular disease.

Several studies have shown that ROS accumulation in the cell activates the autophagy process. For example, a mutation in an antioxidative superoxide dismutase (SOD1) gene modulates autophagy. Reports from different laboratories have described autophagy activation in

transgenic mice expressing mutant SOD1 [61, 62]. In the first report, SOD1^{G93A} transgenic mice displayed inhibition of mTOR and accumulation of lipid-conjugated LC3, the mammalian homologue of Atg8 [62]. A recent report showed that mutant SOD1 interacts directly with p62 (also called SQSTM1), an LC3 binding partner known to target protein aggregates for autophagic degradation. Indeed, this interaction is proposed to mediate autophagic degradation of mutant SOD1 [63]. Ruth Scherz-Shouval's group has suggested two major ROS (H_2O_2 and $\text{O}_2^{\cdot-}$) as the main regulators of autophagy [64]. H_2O_2 is a striking contender for signaling because it is comparatively stable and long-lived as compared to other ROS species. Its neutral ionic state enables it to exit the mitochondria with ease. It has been implicated as a signaling molecule in various signal transduction pathways, including autophagy [65]. Indeed, ATG4, an essential protease in the autophagic pathway, has been identified as a direct target for oxidation by H_2O_2 during starvation [66]. Other studies report autophagy activation in response to exogenous H_2O_2 treatment [67]. In most cases, this treatment leads to oxidative stress and mitochondrial damage, which induce autophagy. Taken together, this evidence supports the vital role of oxidative stress in the induction of autophagy.

7. Redox signaling and autophagy

Redox signaling involves targeted modification by reactive species through a chemically reversible reaction. The reaction of ROS/RNS with the target molecule acts as an on-off switch signal. Oxidative damage in response to oxidative stress leads to irreversible oxidation of proteins lipids and nucleic acids [68]. However, since amino-acid residues in proteins, fatty acids in lipids and nucleic acid bases have different susceptibility to oxidative stress. "Mild" oxidative stress appears to provide selectivity for a specifically targeted molecule and may constitute a signaling mechanism even when an irreversible modification is produced [69]. Oxidative damage can be repaired to a certain extent, as evident in the diverse array of DNA repair systems. In addition, oxidized proteins can be effectively degraded and recycled by both proteasome and autophagy systems. Proteasomal degradation of oxidatively modified proteins requires protein unfolding; thus, only mildly oxidized proteins are suitable proteasome substrates. During oxidative stress, the resulting cellular response and outcome is likely to involve both redox signaling and oxidative damage, whose contribution will depend on the concentration and nature of the ROS/RNS involved [70]. NAC decreased both cellular ROS production and autophagy, implicating redox thiol signaling as an important regulator of autophagy.

8. Autophagy as cell death progression or survival in response to oxidative stress

Similar to autophagy, ROS/RNS formation has been linked to the regulation of both pro-survival and cell death pathways (Figure 1). To generalize, basal levels of ROS/RNS formation and those induced by growth factor receptor activation are essential for maintaining appro-

appropriate cellular homeostasis and mediate cell proliferation by redox signaling [71]. ROS/RNS-mediated redox signaling also regulates survival-promoting adaptive responses to cellular stress. Redox signaling, generally occurs in the absence of an overall imbalance of pro-oxidants and antioxidants [71]. In contrast, when antioxidant defenses are surpassed by ROS/RNS formation, and oxidative damage is not repaired by endogenous mechanisms, oxidative stress leads to cell death. However, although oxidative damage to proteins, lipids, and nucleic acids is associated with activation of programmed cell death, both pro-apoptotic and pro-survival signaling proteins are modulated by specific reversible oxidative modifications [71, 72].

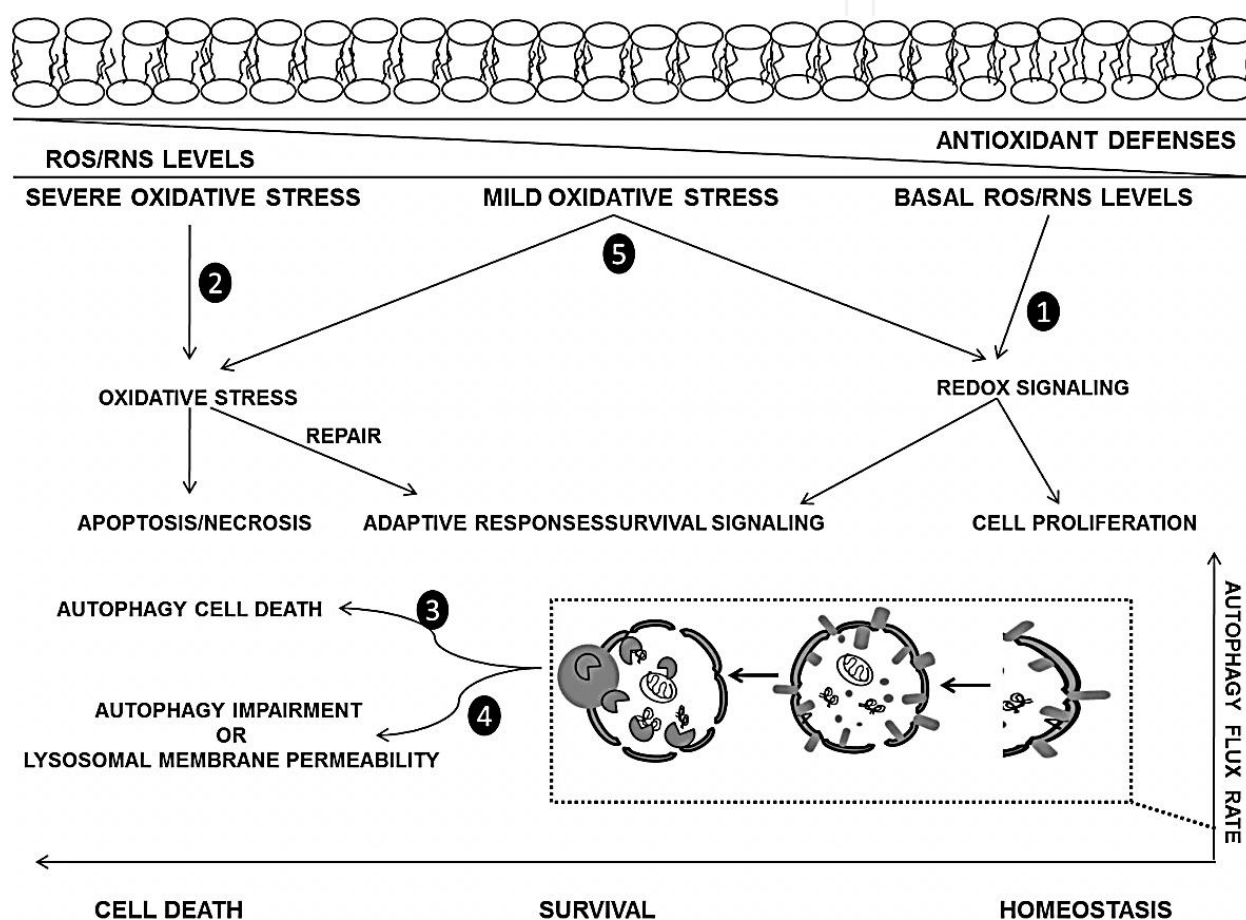


Figure 1. Oxidative stress, redox signaling, and autophagy: cell death versus survival. (1) Basal or physiological levels of ROS/RNS play an important homeostatic role regulating signal transduction involved in proliferation and survival. (2) In contrast, when antioxidant defenses are surpassed by ROS/RNS formation and oxidative damage is not repaired by endogenous mechanisms, oxidative stress leads to cell death. (3) Under these pathological conditions, “excessive” autophagy might promote cell death through degradation of important components within the cell. In addition, (4) lysosomal membrane permeabilization induced by stress can also contribute to cell death. However, (5) “mild” oxidative stress can act as a signaling mechanism leading to adaptive stress responses. Oxidative damage can be repaired to a certain extent and oxidized biomolecules, such as proteins, can be degraded and recycled by distinct processes, including autophagy. During oxidative stress the resulting cellular response and outcome is likely to involve both redox signaling and oxidative damage, whose contribution will depend on the concentration and nature of the ROS/RNS involved, the duration of the stress response, and cell type or gender. A clear distinction between both oxidative stress and redox signaling is hard to define.

9. Modulation of autophagy by antioxidant in autoimmune disease

There is a lack of literature on the role of redox signaling by oxidative cysteine modification in autophagy. Cysteines can act as post-translational modification sites which are utilized for targeting proteins to membranes and/or influence protein activity, localization, and/or protein-protein interactions [73]. In response to ROS or RNS, redox-sensitive cysteines undergo reversible and irreversible thiol modifications. Almost all physiological oxidants react with thiols [74]. O_2^- and peroxides (H_2O_2 , and $ONOO^-$) mediate one- and two-electron oxidation of protein cysteines respectively, leading to formation of reactive intermediates protein sulfenic acids (PSOH) and protein thiyl radicals (PS), respectively. PSOH can lead to formation of additional oxidative modifications that act as signaling events regulating protein function. The reaction of PSOH with either another protein cysteine or GSH will generate a disulfide bond or a glutathionylated residue (PSSG). PSSG is considered as a protective modification against irreversible cysteine oxidation. PSOH can undergo further reaction with H_2O_2 and irreversibly generate protein sulfinic (PSO_2H) and sulfonic (PSO_3H) acids. The reversible covalent adduction of a nitroso group (NO) to a protein cysteine is referred to as protein nitros(yl)ation (PSNO). PSNO occurs by endogenous NO-mediated nitros(yl)ating agents such as dinitrogen trioxide (N_2O_3) or by transition metal-catalyzed addition of NO. The transfer of NO groups between PSNO and GSNO (transnitros(yl)ation) is one of the major mechanisms mediating PSNO. GSNO is formed during oxidation of NO in the presence of GSH, or as a by-product from the oxidation of GSH by $ONOO^-$ [75].

Reversible conjugation of the Atg8 family of proteins to autophagosomal membrane is a hallmark event in the autophagic process. All Atg8 homologues (including LC3) are substrates for the Atg4 family of cysteine proteases. Atg4s cleave Atg8 near the C-terminus downstream of a conserved glycine, enabling its conjugation to PE. Atg4 further cleaves Atg8 (LC3)-PE, releasing it from the membrane. Thus, after initial cleavage of Atg8(LC3)-like proteins, Atg4 must be inactivated to ensure the conjugation of Atg8 (LC3) to the autophagosomal membrane. After the autophagosome fuses with the lysosome, Atg4 is re-activated in order to dilapidate and recycle Atg8 (LC3). Recently, it was revealed that upon starvation, increased generation of mitochondrial H_2O_2 oxidizes and inactivates Atg4 after the initial cleavage of LC3, ensuring structural integrity of the mature form [66]. A number of signaling molecules regulating apoptosis are reported to be regulated by oxidative cysteine modifications. For example, glutathionylation (PSSG) of nuclear factor-kappa B (NF- κ B) and caspases, have been reported to regulate apoptotic cell death [76]. Similarly, caspases and the anti-apoptotic Bcl-2 protein have been shown to be nitros(yl)ated (PSNO) under basal conditions in human lung epithelial cancer cells, and their denitros(yl)ation is necessitated for their activation during apoptosis [77]. Both apoptosis and autophagy are simultaneously activated by the distinct stressors. Cross-talk between both the signaling pathways has been evidenced primarily by (1) interaction of Bcl-2 or Bcl-xl with Beclin-1, which inhibits autophagy; and (2) cleavage/degradation of Beclin-1 by caspases [78]. Thus, both glutathionylation and nitrosylation might exert regulatory roles in autophagy by indirect regulation of Bcl-2 and caspase activity [77]. Protein nitros(yl)ation exert inhibitory effects on autophagy. Nitros(yl)ation and inhibition of JNK1

and IKK β signaling pathways are also reported to inhibit autophagy by increased Bcl-2-Beclin-1 interaction and decreased AMPK phosphorylation [79].

AMPK is a key regulator of metabolism, particularly glycolysis. By regulation of ULK1 and mTORC1 complexes, AMPK has been demonstrated to regulate autophagy [80]. In HEK293 cells, H₂O₂ was recently demonstrated to oxidize cysteine residues of α -(Cys299 and Cys304) and β -subunits of AMPK via glutathionylation, with a concomitant increase in its kinase activity. Hypoxia is reported to activate AMPK via mitochondrial ROS formation independent from the AMP/ATP ratio in mitochondrial DNA-deficient cells [81]. Ataxia-telangiectasia mutated (ATM) protein kinase is activated by DNA double strand breaks (DSBs) to initiate DNA damage response. Cells lacking ATM are also hypersensitive to insults other than DSBs, particularly oxidative stress. Oxidation of ATM directly induces its activation in the absence of DNA damage via a disulfide-cross-linking dimerization [82]. Activation of ATM by oxidative stress or genotoxic damage was recently reported to activate AMPK and the tuberous sclerosis complex 2 (TSC2), which in turn participates in energy sensing and growth factor signaling to repress the kinase mTOR in the mTORC1 complex [83]. Studies regarding mechanisms by which ROS, redox signaling, and autophagy regulate autoimmune disease progression is a new research field that could provide pivotal information toward understanding and development of therapeutic to manage the disease.

10. Modulation of autophagy in systemic lupus erythematosus

SLE is an autoimmune disorder characterized by the auto-antibodies directed against self-antigens, immune complex formation and immune deregulation, resulting in damage to any organ, including kidneys, skin, blood cells, and nervous system [84]. It is a multifactorial disease and its etiology comprises hormonal, environmental and genetic background. While mechanisms underlying this systemic autoimmune response remain largely unknown, several vital studies show that uncontrolled reactive oxygen generation and defect in regulation of antioxidant system are, in part, crucial factors for the pathogenesis of SLE [85]. The uncontrolled oxidative species generations are speculated to be involved in the production, expansion of antibody flares [86] and various clinical features in SLE [87]. Oxidative damage mediated by ROS results in formation of deleterious byproducts, such as aldehydic products, and leads to development of adducts with proteins. The consequence of this effect makes them highly immunogenic, thus inducing pathogenic antibodies in SLE [88]. In the last 2 decades, there has been substantial progress in understanding the mechanism of oxidative stress in SLE pathogenesis (Figure 2) and the level of intracellular glutathione has been regarded as a checkpoint of oxidative stress [1]. Altered signal transduction pathways, mTOR is activated by relative depletion of glutathione and supplementation of N-acetyl cysteine (NAC), a precursor of glutathione. mTOR replenishes intracellular glutathione, inhibits mTOR signaling and diminished oxidative stress mediated damage in SLE [89]. Glutathione is a key cellular component, a small tri-peptide constructed from three amino acids (glycine, glutamic acid and cysteine), known to be a powerful antioxidant. The main function of glutathione is to protect the cell and mitochondria from oxidative damage, indicating its role in energy utilization.

Management of disease through supplement of NAC and rapamycin has shown promise as a therapy for SLE patients. Administration of rapamycin decreased production of autoantibodies, glomerular deposits of immunoglobulins, development of proteinuria, and prolonged survival in murine SLE. Interestingly, autophagy is regulated by mTOR pathway, and mTOR is activated by relative depletion of glutathione. Thus, redox signaling may provide a link between altered autophagy and depletion of glutathione and autophagy regulation by replenishment of intracellular glutathione may have a therapeutic intervention for disease management [90].

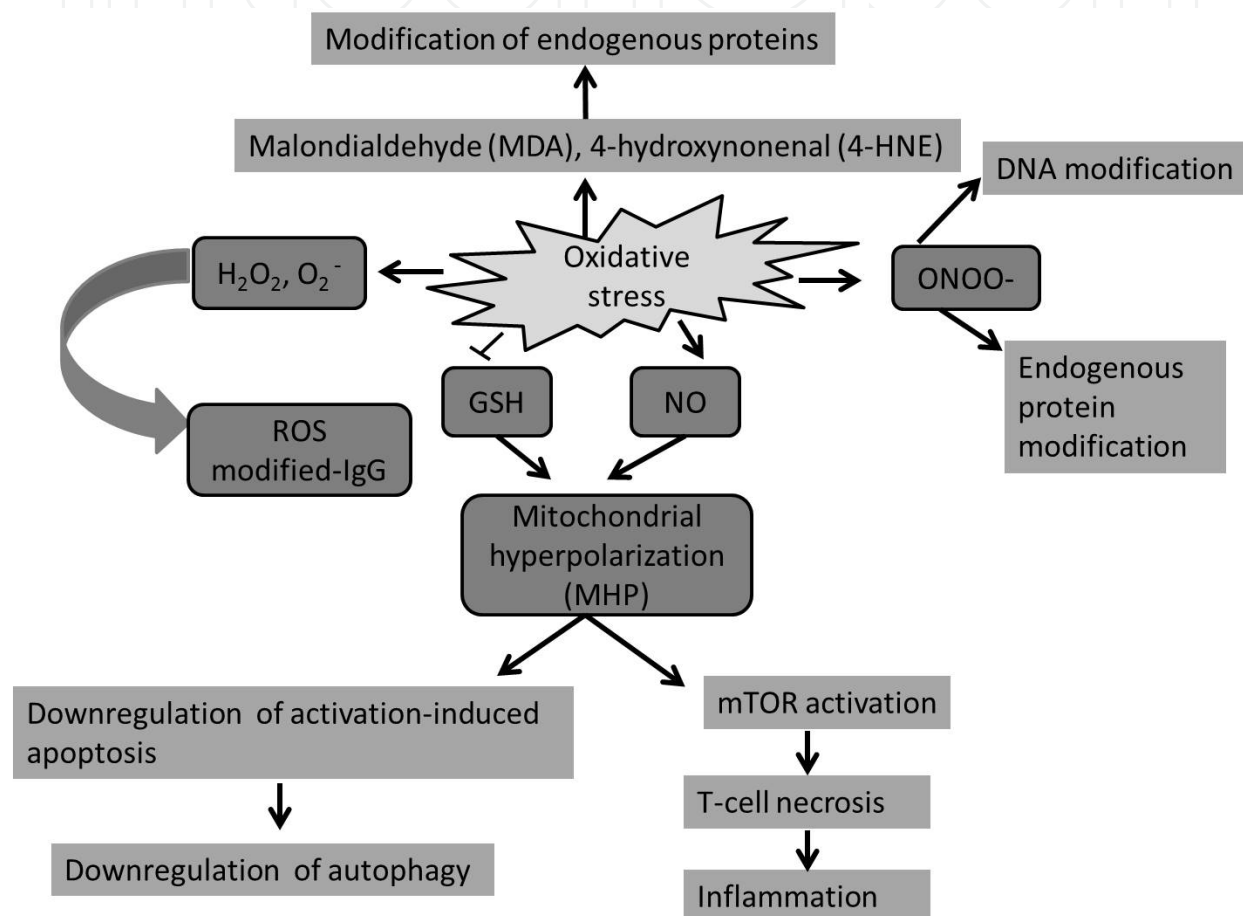


Figure 2. Oxidative stress involvement in the pathogenesis of SLE. MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal; GSH, glutathione; MHP, mitochondrial hyperpolarization; NO, nitric oxide; O_2^- , superoxide; ONOO $^-$, peroxynitrite.

It has been shown that changes in the intracellular redox environment of in cells, through oxidative stress, have been reported to be critical for cellular immune dysfunction [48], activation of apoptotic enzymes, and apoptosis [15]. Decreased intracellular glutathione levels in the various blood components, including total lymphocytes and its subsets (CD4, CD8 T cell), are strongly associated with disease severity and linked to increase Th1/Th2 cytokine imbalance and lymphocyte apoptosis in SLE patients [49]. Similarly, Tewthanom et al. [41] reported that administration of NAC may be beneficial for patients with mild SLE in terms of

decreasing lipid peroxidation. Lai et al. demonstrated that GSH regulates elevation of mitochondrial transmembrane potential ($\Delta\psi_m$) or mitochondrial hyperpolarization (MHP), which in turn activates mTOR in lupus T cells [91]. mTOR skews cell death signal processing, modulates T-cell differentiation, and, in particular, inhibits development of CD4⁺/CD25⁺/Foxp3⁺ regulatory T cells which are deficient in patients with active SLE. These studies are important as they suggest the blockade of mTOR with rapamycin and NAC improves lupus disease activity [89, 91].

In recent years, perturbation in autophagy has been implicated in a number of diseases, including SLE [8]. Towns et al. [92] found that serum factors, likely autoantibodies, purified from SLE patients were able to induce autophagy in neuroblastoma cell lines, providing a further link between autophagy and SLE. Several other groups have reported activated autophagy pathway in T and B lymphocytes as a mechanism for survival of autoreactive T and B lymphocytes. Inhibition of autophagy by blocking mTOR signaling has been suggested as a novel target for treatment in this disease. Importantly, Lai et. al. has shown that blockade of mTOR with supplementation of NAC reversing glutathione depletion and improving disease activity and fatigue in SLE patients [91]. NAC treatment promotes expansion of CD4⁺CD25⁺FOXP3⁺ T cell subsets and inhibits anti-DNA antibody production. Indeed, NAC reversed expansion of CD4⁺CD8⁺ T cells, which exhibited the most prominent mTOR activation before treatment with NAC, and may be responsible for promoting anti-DNA autoantibody production by B cells. They showed that NAC acts as a sensor of $\Delta\psi_m$, mTOR governs T-cell signaling events implicated in pathogenesis [91]. Since, activation of autophagy has been considered to be principally regulated by the mTOR pathway, supplementation of NAC block mTOR signaling in SLE patients.

11. Conclusion

The autophagic process is highly regulated and is stimulated by several factors including oxidative stress. Cell death occurs when over production of ROS/NOS fails to be corrected by antioxidant machinery, activate autophagy, which in turn removes damaged components, or when damage exceeds a certain threshold. However, whether autophagy leads to a pro-survival response or cell death depends on the situation and severity of oxidative stress occurring in a particular pathologic setting. Evidence from genetics, cell biology and lupus animal model studies suggests a pivotal role of autophagy in mediating occurrence and development of SLE. Importantly, autophagy is regulated by the mTOR pathway, and mTOR is activated by relative depletion of glutathione. This suggests that redox signaling may provide a link between altered redox signaling and autophagy in SLE. Therefore it will be interesting to study the effect of therapeutic supplements of NAC on autophagy in animal models of lupus and in SLE patients. Such controlled clinical studies encourage exploration of the therapeutic potential of NAC, which might prove to provide an inexpensive and significant alternative therapy for SLE.

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